

Aberrations in the cross-talks among redox, nuclear factor- $\kappa$ B and Wnt/catenin pathway signaling underpin Myalgic Encephalomyelitis and chronic fatigue syndrome: a review and new hypothesis based on results of network, enrichment and annotation analyses.

Michael Maes, M.D., Ph.D.<sup>a,b,c</sup>, Marta Kubera<sup>d</sup>, Magdalena Kotańska, Ph.D.<sup>c</sup>

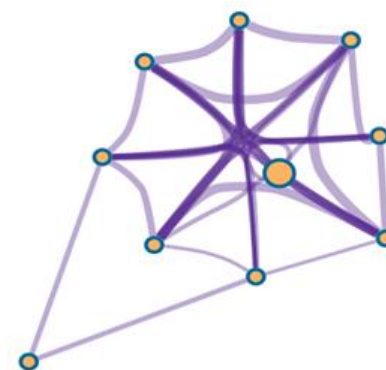
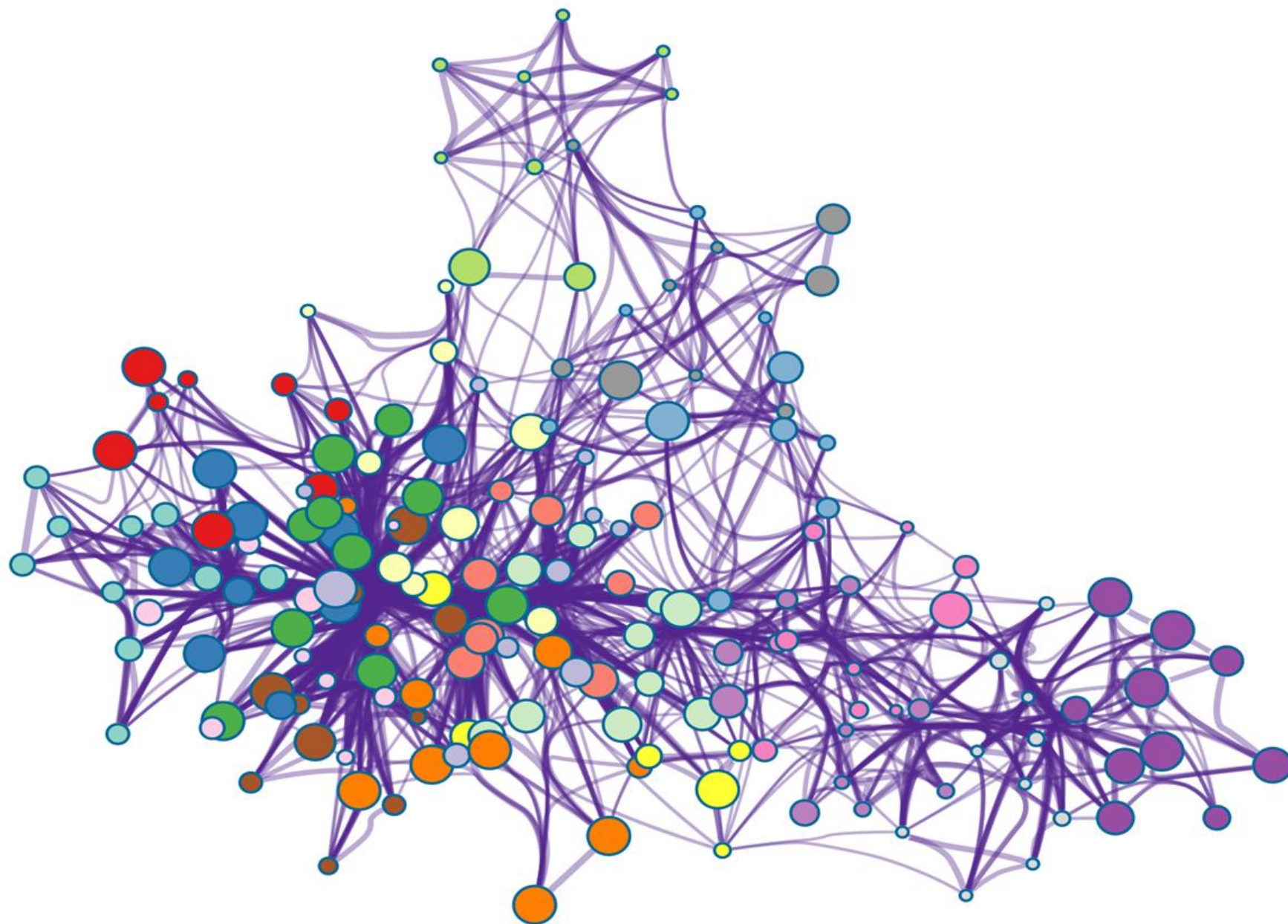
<sup>a</sup> Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

<sup>b</sup> Department of Psychiatry, Medical University of Plovdiv, Plovdiv, Bulgaria

<sup>c</sup> IMPACT Strategic Research Center, Deakin University, Geelong, Australia

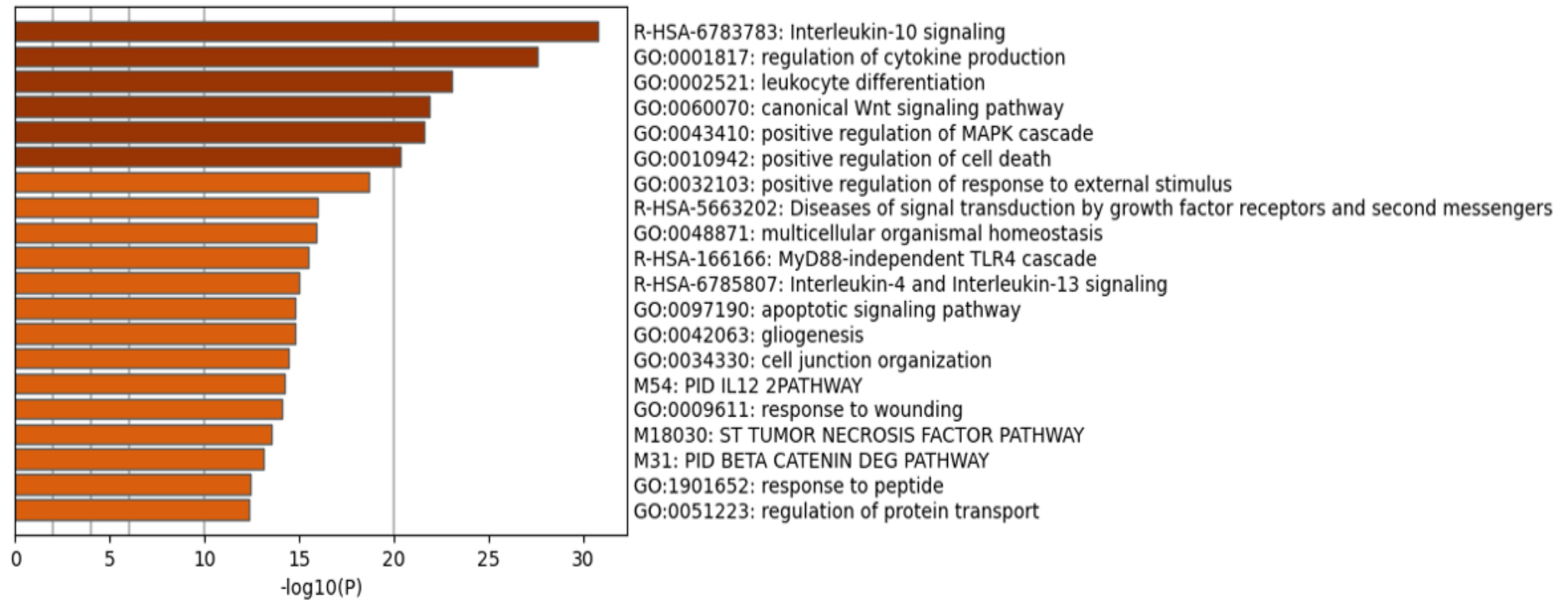
<sup>d</sup> Laboratory of Immunoendocrinology, Department of Experimental Neuroendocrinology, Maj Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna St., 31-343 Crakow, Poland

<sup>e</sup> Department of Pharmacological Screening, Jagiellonian University, Medical College, Medyczna 9, PL 30-688 Cracow, Poland

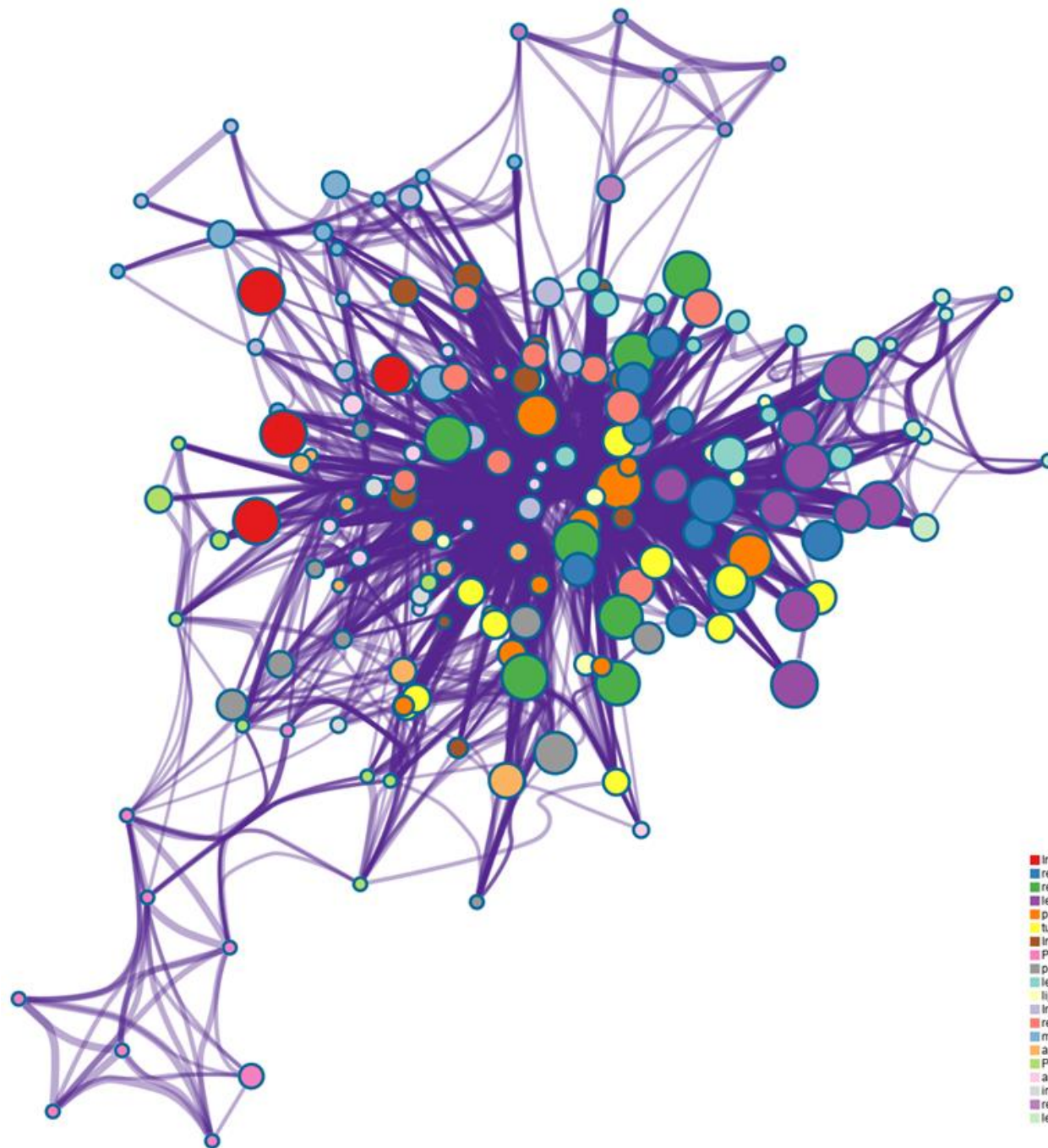


- Interleukin-10 signaling
- regulation of cytokine production
- leukocyte differentiation
- canonical Wnt signaling pathway
- positive regulation of MAPK cascade
- positive regulation of cell death
- positive regulation of response to external stimulus
- Diseases of signal transduction by growth factor rece
- multicellular organismal homeostasis
- MyD88-independent TLR4 cascade
- Interleukin-4 and Interleukin-13 signaling
- apoptotic signaling pathway
- gliogenesis
- cell junction organization
- PID IL12 2PATHWAY
- response to wounding
- ST TUMOR NECROSIS FACTOR PATHWAY
- PID BETA CATENIN DEG PATHWAY
- response to peptide
- regulation of protein transport

**ESF 2, Figure 1.** Enriched ontology term clusters in the chronic fatigue spectrum disorders network. Terms are represented by a circle node, with the colors representing cluster identity and their size reflecting the number of input genes. One term is used to describe the clusters (colored labels). The thickness of the (bundled) edges linking the terms reflects the similarity score (threshold is  $> 0,3$ ). The network is constructed and visualized using MetaScape and Cytoscape (v3.1.2)



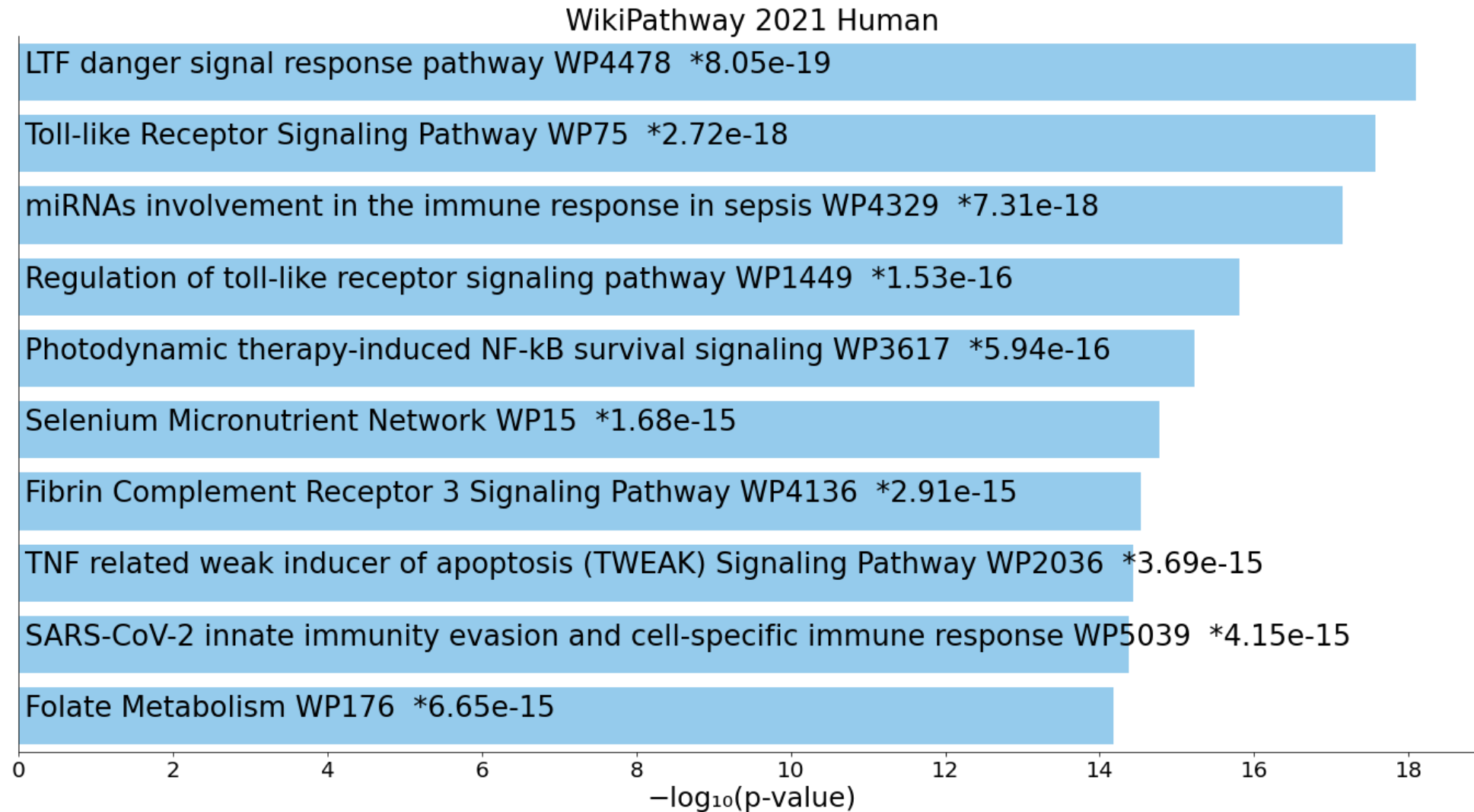
**ESF 2, Figure 2.** Heatmap of enriched ontology clusters showing the top-20 functions that were overexpressed in the expanded network of patients with chronic fatigue spectrum disorders (accumulative hypergeometric p-values)



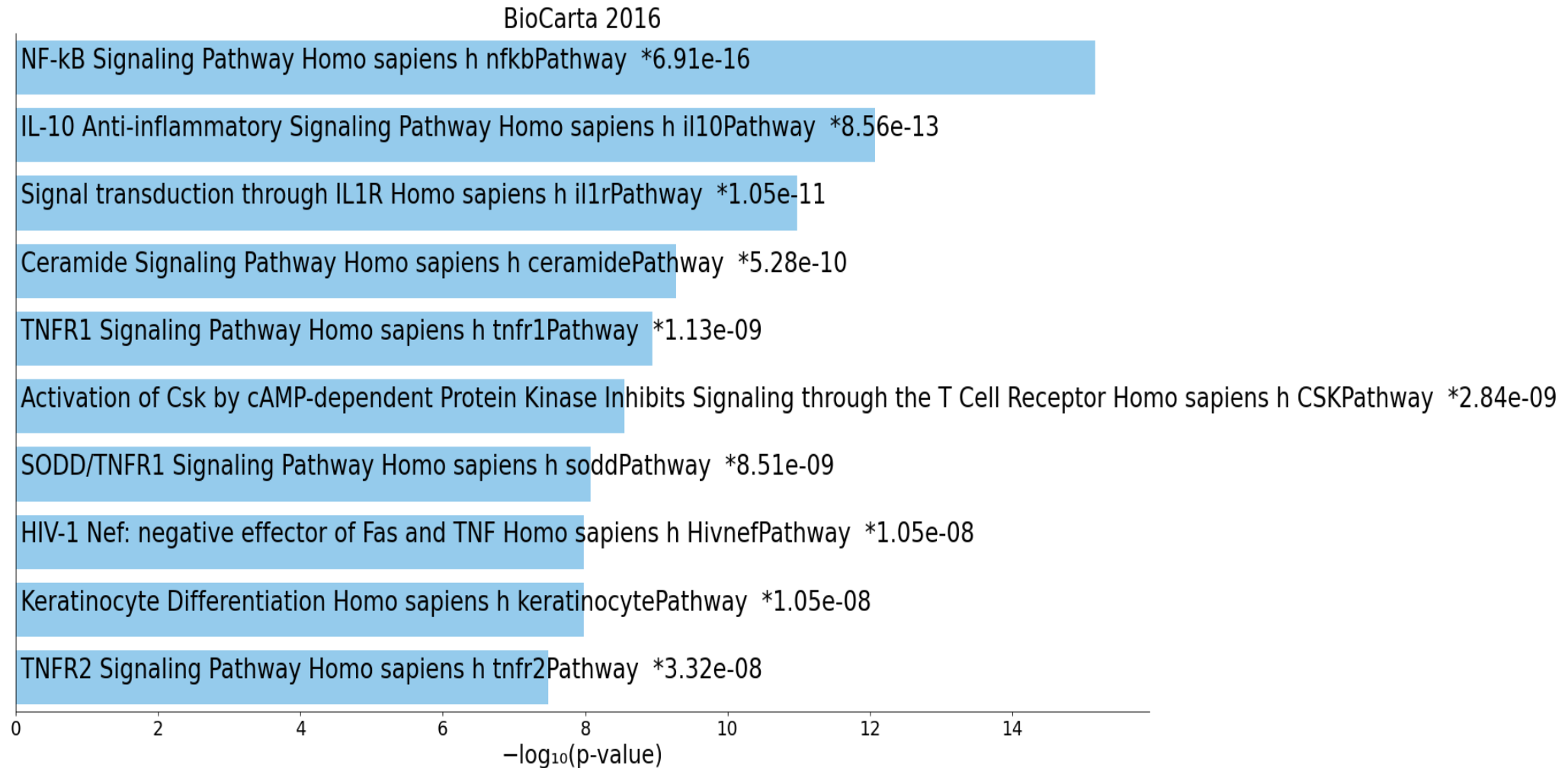
- Interleukin-10 signaling
- regulation of cytokine production
- response to lipopolysaccharide
- leukocyte mediated immunity
- positive regulation of response to external stimulus
- tumor necrosis factor production
- Interleukin-4 and Interleukin-13 signaling
- PID IL12.2PATHWAY
- positive regulation of cell death
- leukocyte migration
- lipopolysaccharide-mediated signaling pathway
- Interleukin-1 signaling
- regulation of secretion
- multicellular organismal homeostasis
- apoptotic signaling pathway
- PID IL23 PATHWAY
- acute inflammatory response
- inflammatory response to antigenic stimulus
- response to wounding
- leukocyte activation involved in immune response



**ESF 2, Figure 3.** Enriched ontology term clusters in the immune subnetwork of chronic fatigue spectrum disorders. Terms are represented by a circle node, with the colors representing cluster identity and their size reflecting the number of input genes. One term is used to describe the clusters (colored labels). The thickness of the (bundled) edges linking the terms reflects the similarity score (threshold is  $> 0.3$ ). The network is constructed and visualized using MetaScape and Cytoscape (v3.1.2)

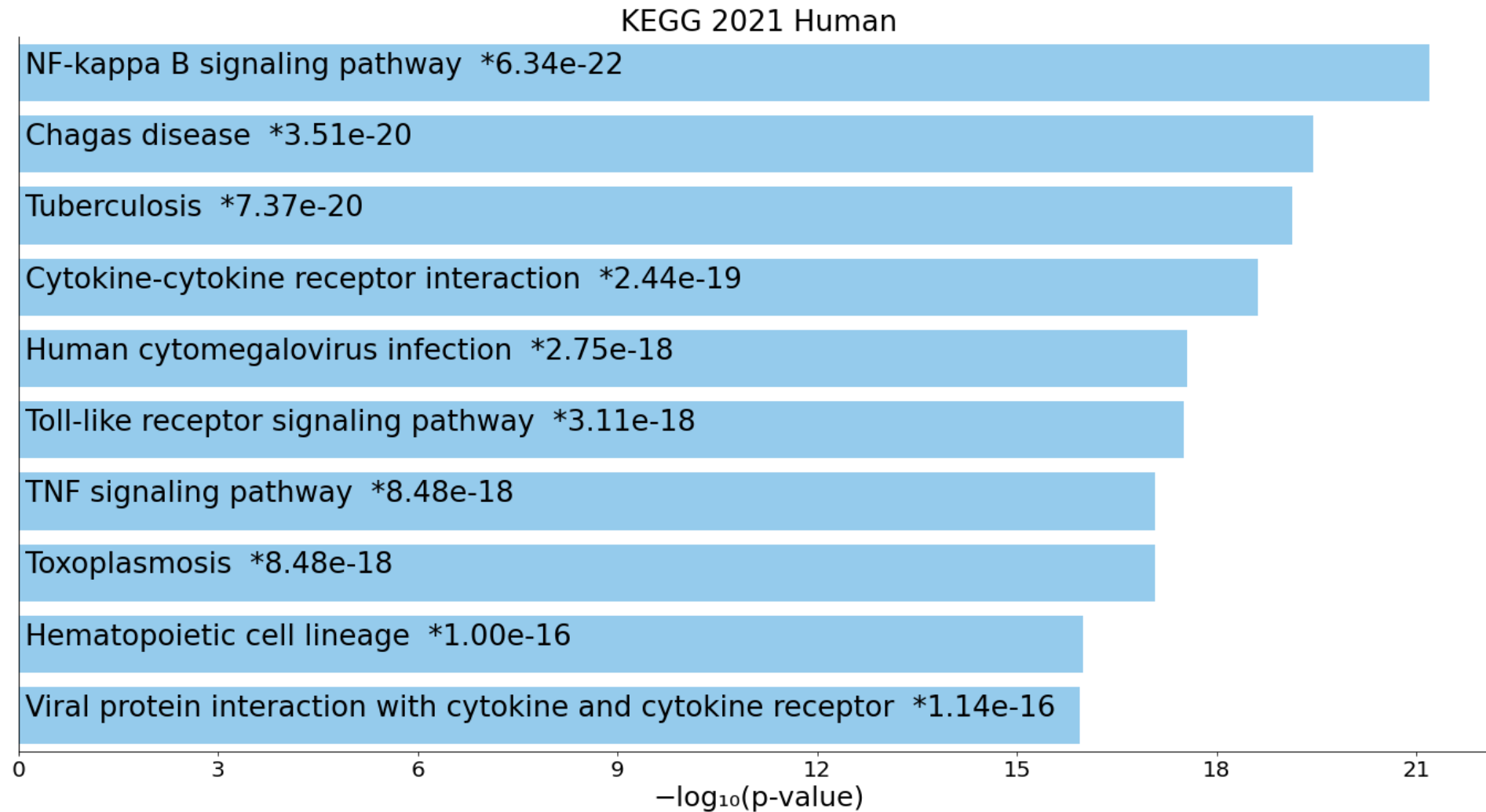


**ESF 2, Figure 4.** Heatmap (top-10) of the enriched WikiPathway terms accumulated in the differently expressed proteins of the immune subnetwork of chronic fatigue spectrum disorders

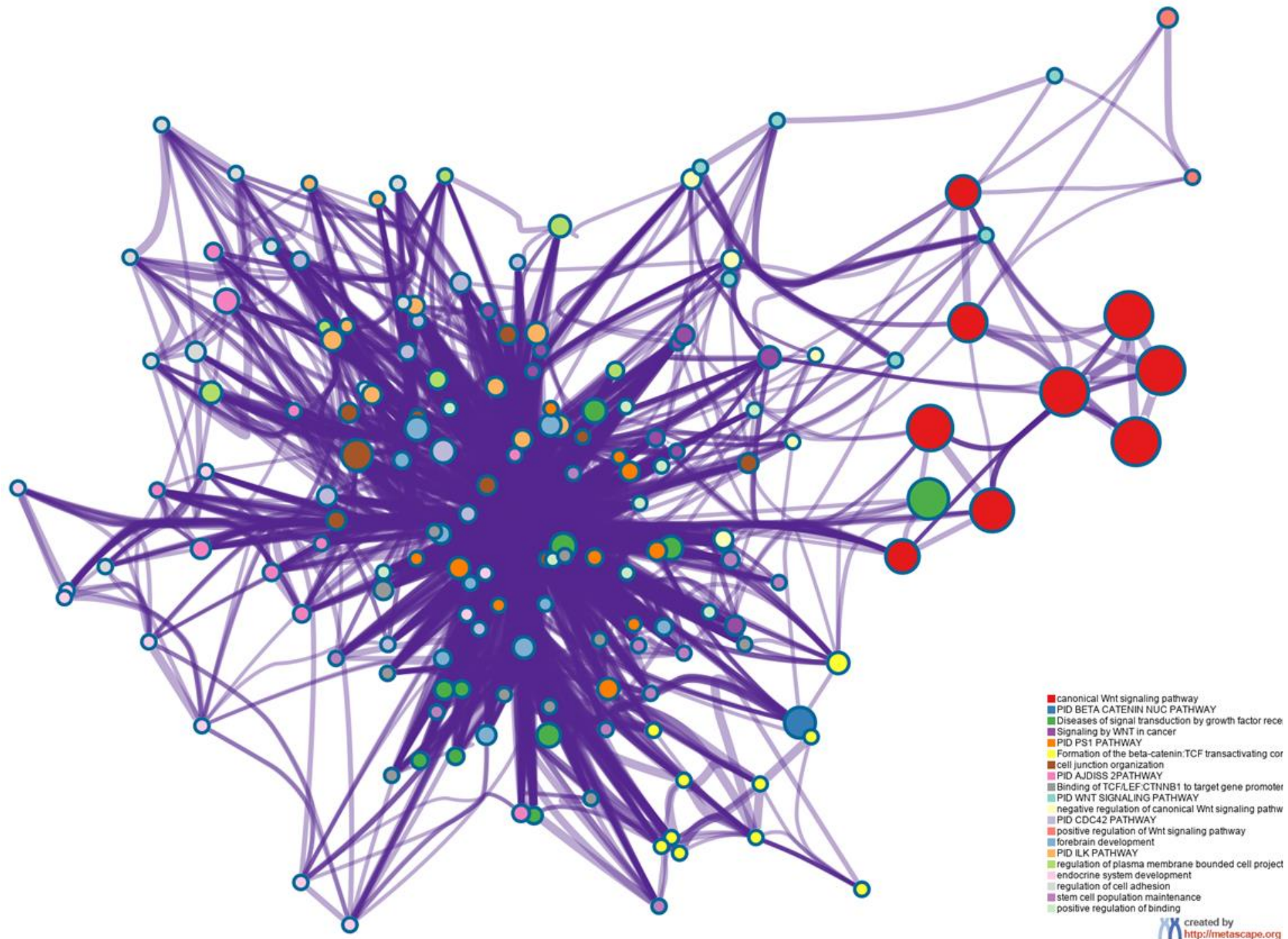


**ESF 2, Figure 5.** Heatmap (top-10) of the BioCarta terms accumulated in the differently expressed proteins of the expanded immune subnetwork of chronic fatigue spectrum disorders

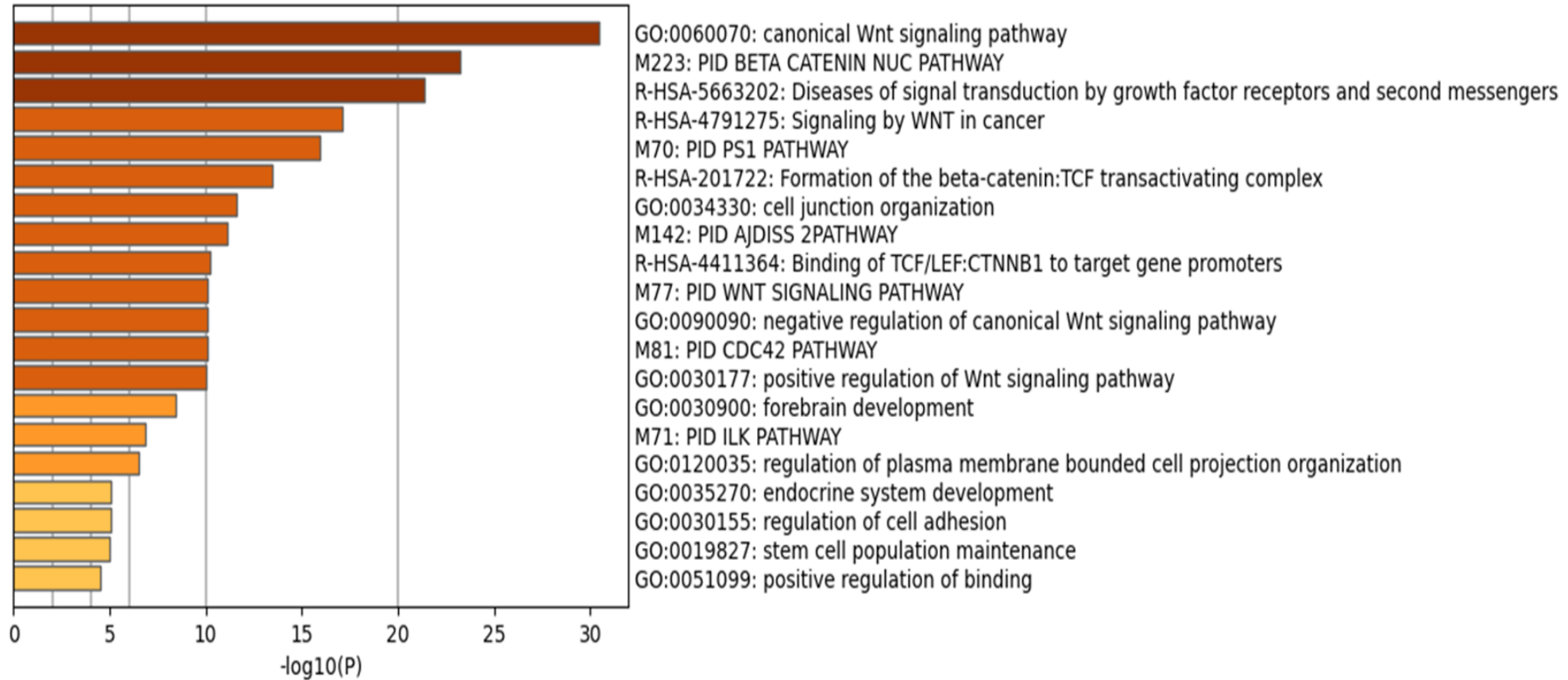




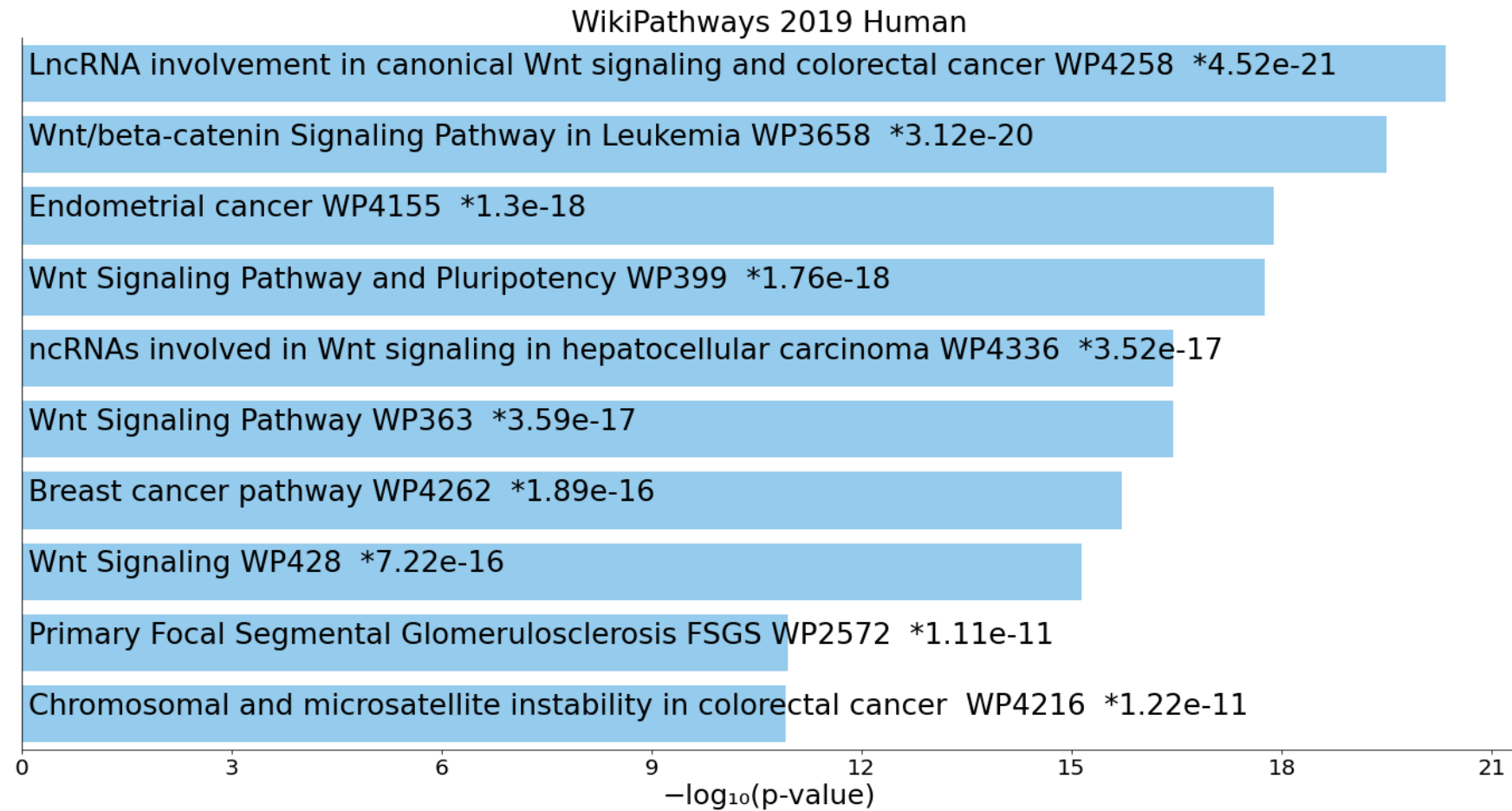
**Figure 6.** Heatmap (top-10) of the KEGG pathways accumulated in the differently expressed proteins of the expanded immune subnetwork of chronic fatigue spectrum disorders



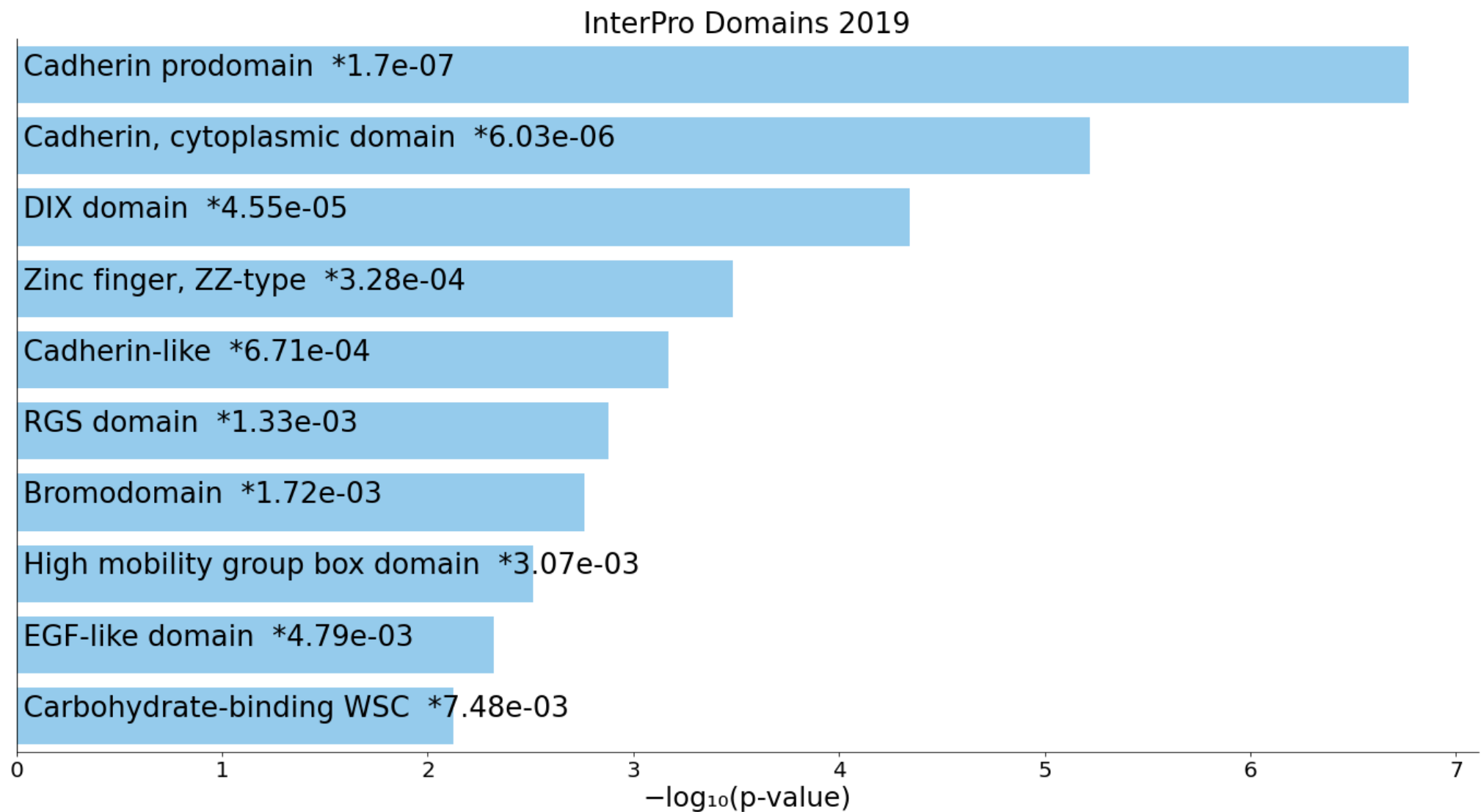
**ESF 2, Figure 7.** Enriched ontology term clusters in the Wnt/catenin subnetwork of chronic fatigue spectrum disorders. Terms are represented by a circle node, with the colors representing cluster identity and their size reflecting the number of input genes. One term is used to describe the clusters (colored labels). The thickness of the (bundled) edges linking the terms reflects the similarity score (threshold is  $> 0.3$ ). The network is constructed and visualized using MetaScape and Cytoscape (v3.1.2).



**ESF 2, Figure 8.** Heatmap of enriched ontology clusters showing the top-20 functions that were overexpressed in the Wnt/catenin subnetwork of patients with chronic fatigue spectrum disorders (accumulative hypergeometric p-values)

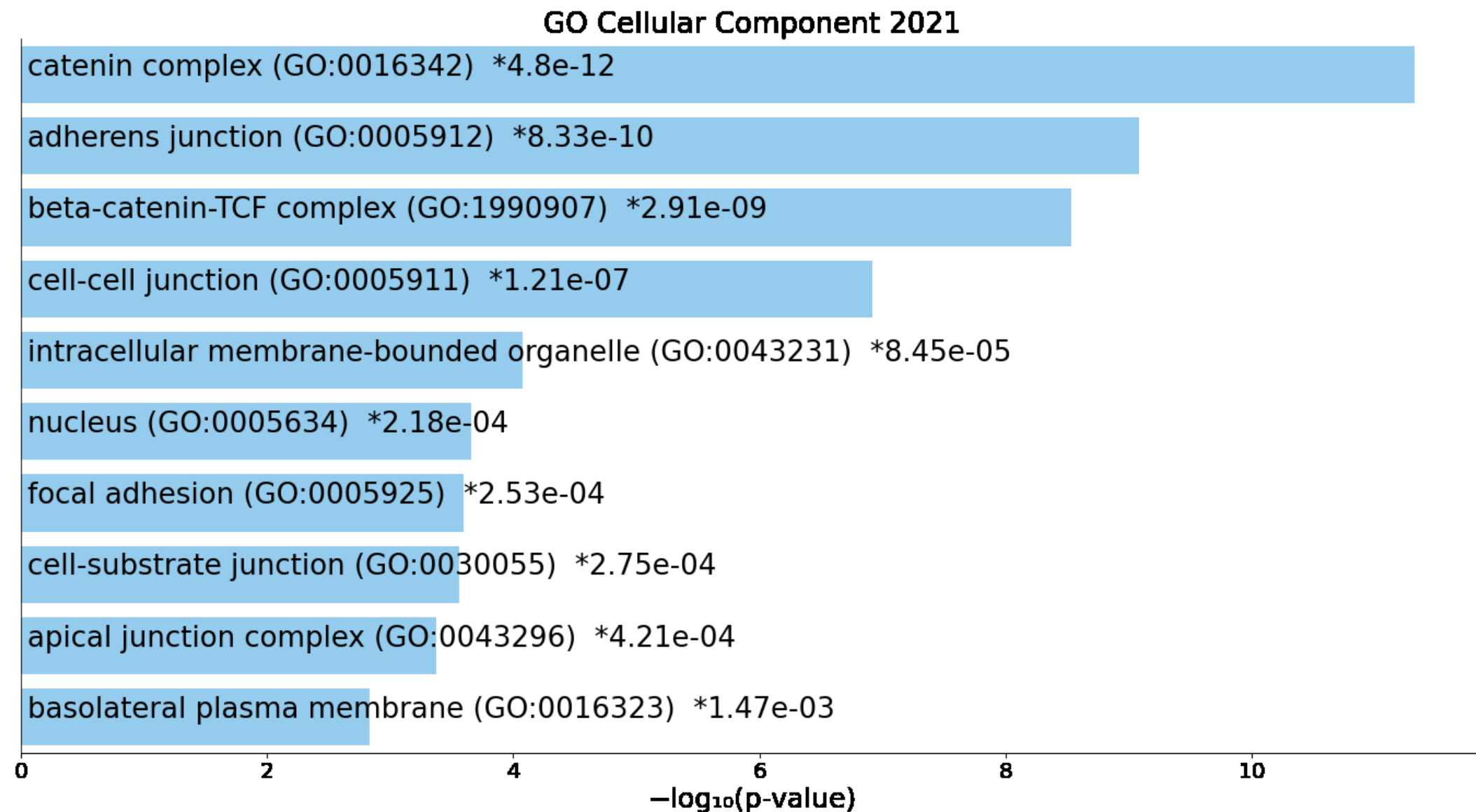


**ESF 2, Figure 9.** Heatmap (top-10) of the WikiPathways accumulated in the differently expressed proteins of the expanded Wnt subnetwork of chronic fatigue spectrum disorders

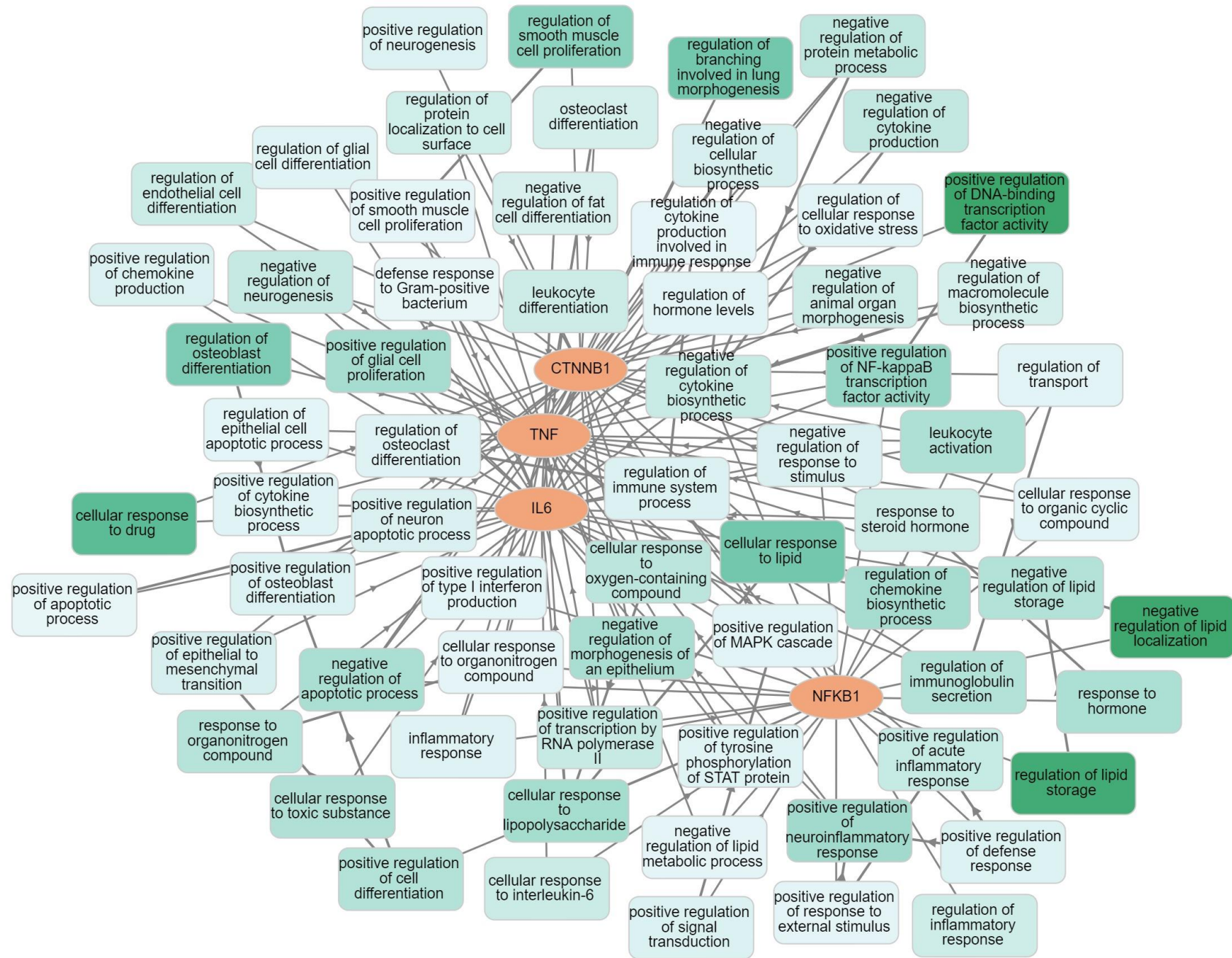


**ESF 2, Figure 10.** Heatmap (top-10) of enriched InterPro domain terms accumulated in the differently expressed proteins of the Wnt/catenin subnetwork of chronic fatigue spectrum disorders



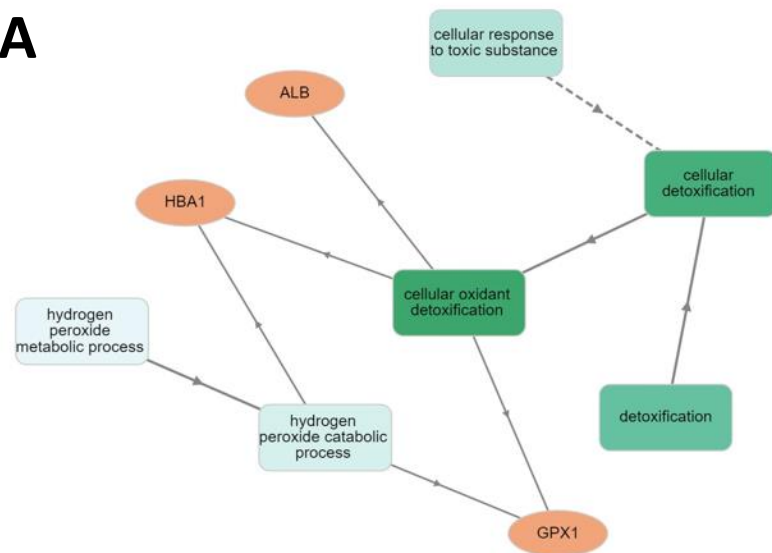


**ESF 2, Figure 11.** Heatmap (top-10) of enriched cellular component terms accumulated in the differently expressed proteins of the Wnt/catenin subnetwork of chronic fatigue spectrum disorders

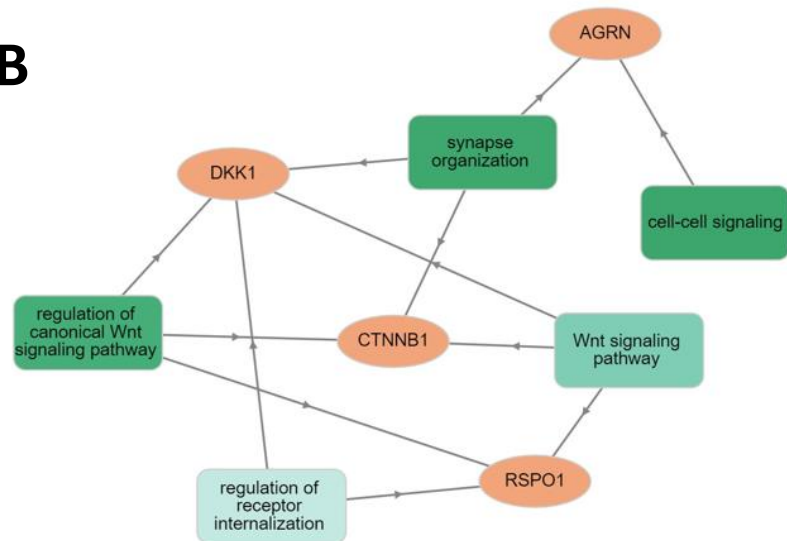


**ESF 2, Figure 12.** Results of GOnet annotation visualization in chronic fatigue spectrum disorders depicting the hierarchical structure of GO terms and the hubs and master regulatory transcription factor

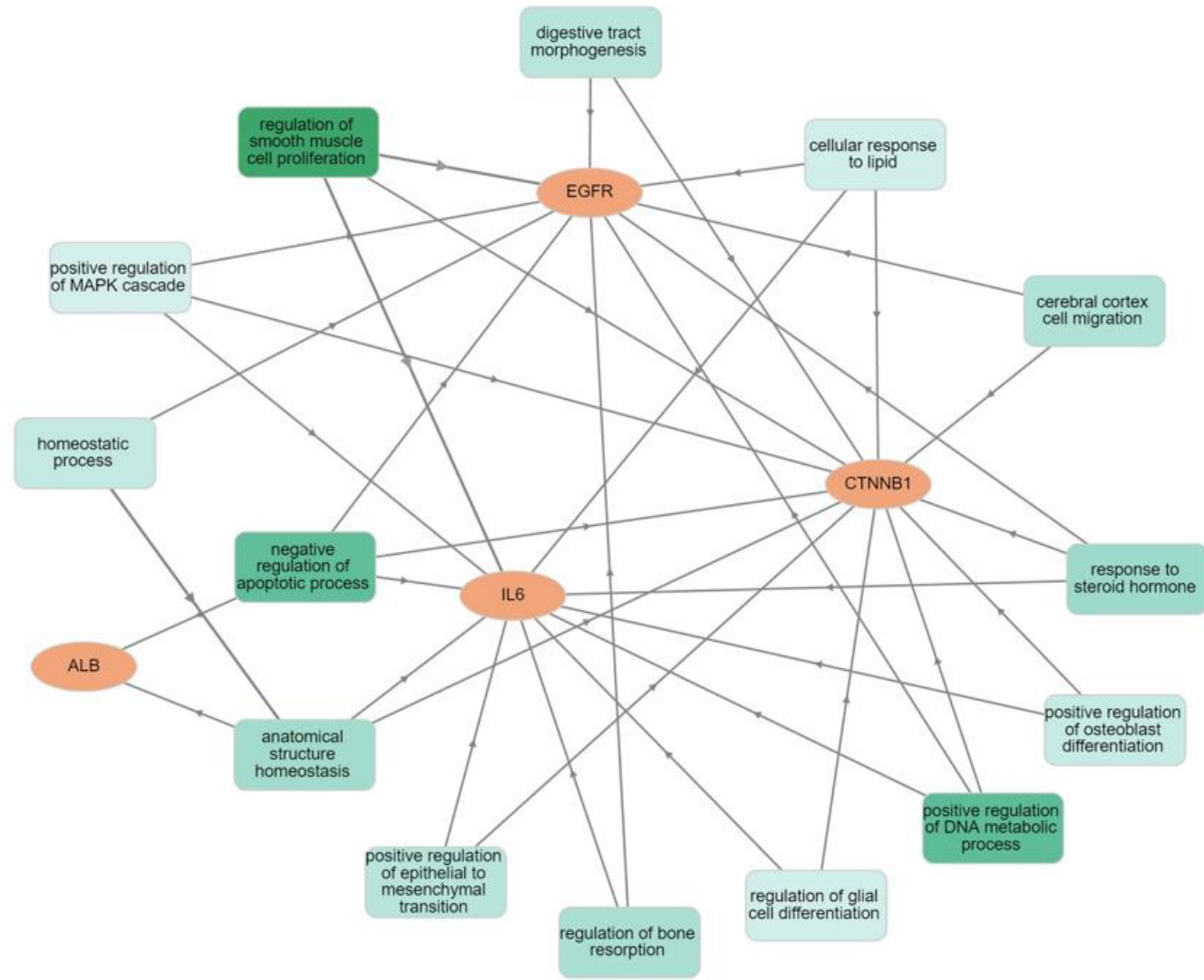
A



B

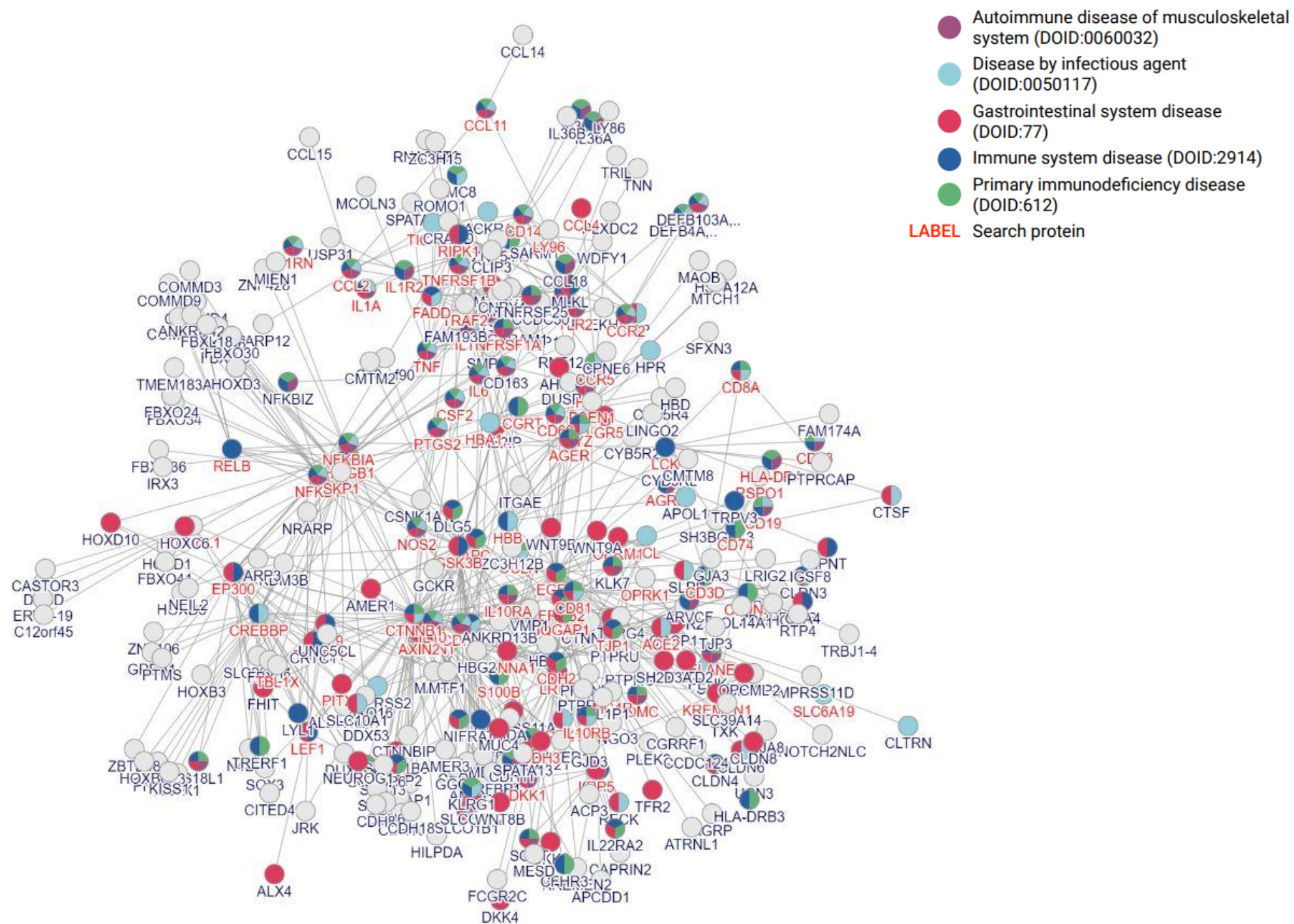


C



**ESF 2, Figure 13.** Results of GOnet annotation visualization in chronic fatigue spectrum disorders depicting the hierarchical structure of GO terms and A: downregulated seed genes; B: seed genes of the Wnt/catenin pathways; and C: the hotspots in STRING enlarged networks







**ESF 2, Figure 14.** An extended network constructed with inBio Discover showing the top Disease Ontology (DOID) annotations of chronic fatigue spectrum disorders